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Abundance of Associated Arbuscular Mycorrhizal Fungi with Pioneer Plants in Affected Area by Mount Merapi Eruption

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Abstract

Arbuscular mycorrhizal fungi (AMF) with mutualism symbiosis with plants are associated with the Fabaceae family's pioneer plants. This study aims to determine the percentage of AMF in the roots and the AMF spore's abundance in the rhizosphere of pioneer plants Calopogonium mucunoides Desv. and Vigna unguiculata (L.) Walp in Mount Merapi National Park after the 2010 eruption. The methods used for root infection analysis were slide methods and root staining, while spore analysis was wet sieving methods. The results showed the highest percentage of AMF infection in C. mucunoides root was found in an area with moderate damage. The highest percentage of AMF infection in V. unguiculata was found in an area with minor damage. The highest number of spores in the two plants' rhizosphere soil was found in an area with minor damage with an average of 10.4 and 9. The levels of damage by Mount Merapi's eruption did not significantly affect the percentage of AMF infection and the number of spores. Glomus dominates all study sites in both plants. Soil organic carbon is one of the abiotic factors correlated with the number of spores in rhizosphere soil.

Keywords: *calopogonium, Merapi, mycorrhizal, succession, vigna*

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Introduction

Forest damage due to land-use change and natural disturbances can reduce the abundance of microorganisms in the soil (Suriadikarta et al., 2011). Soil microorganisms influence the plant community's structure above the soil surface (Horn et al., 2017). Arbuscular mycorrhizal fungi (AMF) are the essential microscopic fungi in the soil (Gao et al., 2019), which have mutualism in 70% to 90% of plants (Mulyana & Asmarahman, 2012; Taylor et al., 2017). AMF, as an obligate parasitic microorganism, will absorb nutrients from the roots of the host plant through its internal hyphae (Talanca, 2010; Munarti et al., 2018). In contrast, external hyphae help increase the nutrition uptake of nitrogen (N) and phosphorus (P) to the host plant (Karti et al., 2018), expand the water absorption area (Suharno et al., 2014), and increase plant tolerance to heavy metals in the soil (Dewi et al. 2014; Ferrol et al., 2016). AMF, which dominates the most terrestrial ecosystems (Dell, 2002), can provide other ecological function such as protecting vegetation against drought and water stress (Chitarra et al., 2016), protection from soil pathogens, increasing plant diversity (Dodd, 2000), and increasing the survival rate of plants in critical land (Ulfa et al., 2006; Suharno & Sancayaningsih, 2013 Saputri & Suwirmen, 2016). AMF plays an essential role for the ecosystem services and land management in the natural and agricultural ecosystem (Barea et al., 2011; Chen et al., 2018) by inoculating specific AMF on pioneer plants or managing indigenous AMF to optimize vegetation communities' growth (Syamsiyah et al., 2014; Cely et al., 2016; Putra et al., 2020). AMF external hyphae with a diameter of 2–5 µm can penetrate soil pores that cannot be penetrated by root hairs with a diameter of 10–20 µm (Pujiastuti, 2018). AMF spores, commonly found in the rhizosphere layer or around the roots of host plants (Sukmadi, 2013), will be stimulated by strigolactones from the roots exudation to germinate to form hyphal branches (Yurisman et al., 2015). AMF is recognized by spores, internal hyphae, external hyphae, and other structures, namely the arbuscular and vesicles (Muryati et al., 2016).

Mount Merapi is the most active volcano of Indonesia's 129 volcanoes (Newhall et al., 2000). The big eruption in 2010 was the most significant in the last 150 years (Marfai et al., 2012; Voight et al., 2000). According to Gunawan et al. (2013) and Marhaento and Kurnia (2015), the pyroclastic flow that spread as far as 13 km with differences in intensity has caused forests in the Mount Merapi National Park (MMNP) to be divided into three damage types: heavy damage (vegetation and soil substrate destroyed), moderate damage (sixty percent of vegetation burned), and minor damage (forests were only exposed to volcanic dush). After ten years of eruption, the forests in MMNP began to carry out succession with high stands in various areas (Gunawan et al., 2013; Afrianto et al., 2016; Parwati et al., 2019; Sutomo, 2019). However, despite there is an association of AMF with

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stands, these are still underreported. The presence of endo mycorrhizae has been published exactly one year after the 2010 eruption, where only 3 out of 12 pioneers were identified to be associated with the genus *Acaulospora, Gigaspora, Glomus* (Agus & Wulandari, 2012). According to Klironomos et al. (2001) and Kivlin et al. (2011), AMF is distributed in an almost terrestrial ecosystem with variations in latitude and longitude, where temperature and humidity affect the dominance of AMF taxa in each location. Stands from the Fabaceae family began to dominate the Merapi forest with severe damage because they were tolerant with acidic soil conditions (Purwanto, 2007; Mensah, 2015) and hot substrates from the eruption (Sutomo, 2019). According to Requena et al. (2001), Pahan (2006), and Belay et al. (2013), the Fabaceae family's ability to survive in critical land is a result from mutualism with AMF, which will increase phosphorus uptake, water and increase fixation.

The succession of vegetation above the soil surface is thought to be correlated with an increase in the abundance of soil microorganisms (Suriadikarta et al., 2011). The magnitude of the disturbance at the beginning will affect the succession (Sutomo, 2019; Utami et al., 2021a; Utami et al., 2021b). The development of the AMF number both infecting pioneer plants and soil at various post-eruption damage levels is interesting to be studied as a preliminary investigation into soil microorganism succession development. Pioneer plants from the Fabaceae family, such as *Calopogonium mucunoides* Desv. and *Vigna unguiculata* (L.) Walp. were chosen because they are a local plant in the MMNP forest area throughout the affected area (Hariani & Erlita, 2016; Muryati et al., 2017). Both plants are mycotrophic species that quickly produce biomass and are commonly used for phytoremediation in degraded areas (Khan, 2005; De-Souza et al., 2012). These two pioneer plants are also classified as legume cover crops (LCC) (Sajimin et al., 2017), which can reduce soil temperature, improve soil fertility, and control weeds (Solomon et al., 2014). This study aimed to determine the percentage of AMF infection in the roots and spore's abundance in the rhizosphere of the pioneer plant *C. mucunoides* Desv. and *V. unguiculata* (L.) Walp in varying levels of damage found in MMNP. This research is essential to identify the specific AMF associated with pioneer plants that developed during the succession in MMNP. The benefits of this research can add information to the presence of AMF in MMNP ecosystem rehabilitation processes by planting local pioneer plants from locations with the most optimum AMF abundance.

Methods

Location determination and sampling This research was conducted from October 2019 to March 2020, approximately ten years after the major eruption of Mount Merapi in 2010. Samples were taken from MMNP in the Special Region of Yogyakarta Province at Sleman Regency (Figure 1). The sampling location is in the MMNP forest, which was affected by the 2010 Merapi eruption with the level of heavy damage at point H (S07°34.689' E110°26.560'), the level of moderate damage at point Mo (S07°35.840' E110°26.120'), and the level of minor damage at point Mi (S07°35.005' E110°24.961') (Figure 1). According to the MMNP forest damage map distribution, the sampling location was determined after the 2010 Merapi eruption (BTNGM, 2011; Marhaento & Kurnia, 2015). Five stands or samples of plants included roots, stems, leaves of *C. mucunoides,* and *V. unguiculata* were taken randomly in the plot of 20 m × 100 m in each area with heavy, moderate, and minor damage. Five samples of the rhizosphere under the stands were taken (0–5 cm away from the stand with a depth of 0–20 cm) (Muryati et al., 2016). Both the stands and the rhizosphere soil were wrapped in aluminum foil and plastic and observed in the Laboratory of Microbiology, Universitas Ahmad Dahlan Yogyakarta. Coordinate points and abiotic parameters,

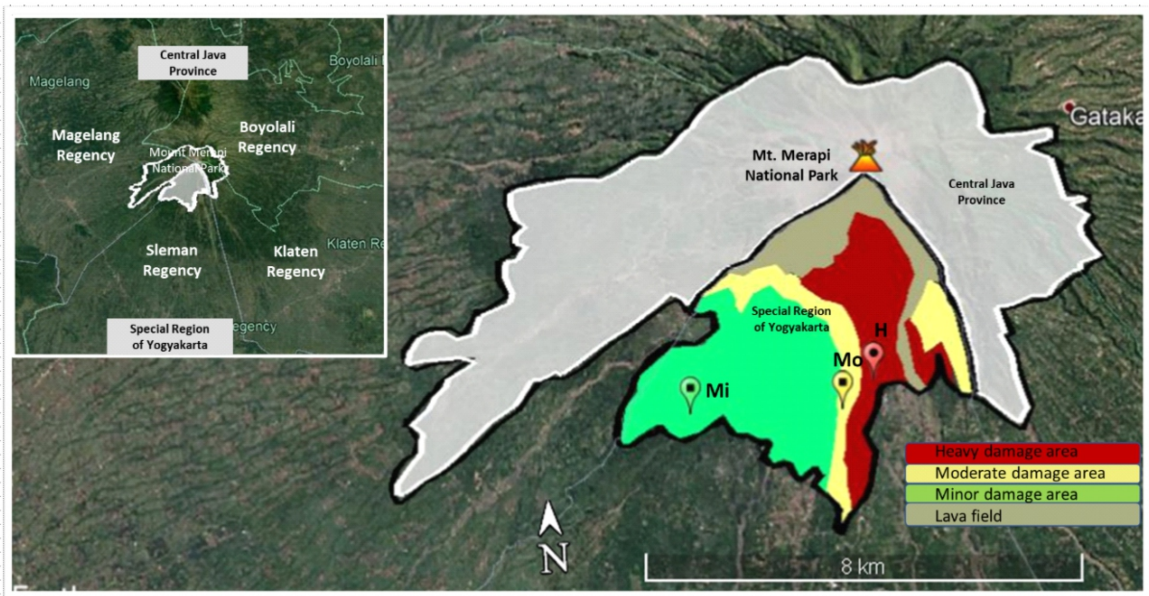

Figure 1 Sampling location in MMNP based on the distribution of MMNP forest damage map after the 2010 Merapi eruption.

including soil temperature, soil pH, soil moisture, soil profile, soil texture, slope, carbon organic soil, light intensity, and rainfall, were also measured in the plot (Syarif et al., 2007; Syib'ili et al., 2013).

AMF monitoring of root infection The methods used for root infection analysis were slide methods and root staining. Clean root samples were fixed with FAA solution for 1 hour to maintain cell and tissue shape (Suharno et al., 2014). The root cells were then immersed in 10% KOH solution for 24 hours, soaked with 1% HCl for 24 hours, and washed with distilled water to color the cells to be observed. Root samples were cut into pieces 1 cm in length (slide method), stained using lactophenol cotton blue for 24 hours (Abdel-fattah, 2001; Marques et al., 2013), dripped with lactoglycerin (destaining), and stored for 24 hours. Ten pieces of roots from each sample were observed by microscope. The presence of AMF was indicated by external hyphae, internal hyphae, vesicles, arbuscular, or spores. The colonization of AMF in roots could be calculated based on the number of all roots observed (Munarti et al., 2018) with the following formula as shown in Equation *[1]*.

$$\text{Percentage of root infections} = \frac{\text{Number of infected roots}}{\text{The number of all roots observed}} \times 100\% \quad [1]$$

Observation of AMF spores in soil samples Spore analysis was carried out by wet sieving methods, which filtering the spores were assisted by water to separate them from the soil. A total of 20 g of soil samples and 100 mL of water were stirred and allowed to stand for 5 minutes. Soil samples were filtered in a stratified sieve set (sizes of 500 μm, 250 μm, 149 μm, and 63 μm respectively from top to bottom) with running water. The suspension held in the lower sieve was rinsed and collected in a jam bottle, then transferred to a microscope slide to be observed under a microscope, and its amount was counted (Suharno et al., 2014). According to Kivlin (2020), most of the AMF spores have a size between 60 μm to 150 μm, so that the spores in the rhizosphere of the two pioneer plants are expected to be optimally identified. Identification of AMF spores found in the rhizosphere is used to identify the types of AMF to the genus taxon. Validation of AMF spores was carried out by comparing with the AMF spore collection database on the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) website.

Data analysis Data were analyzed quantitatively through statistical tests to determine the ratio of the abundance of AMF in the areas affected by Mount Merapi's eruption with heavy, moderate, and minor damage levels. Data were analyzed to determine the correlation between the abiotic parameters, the percentage of infection, and the number of AMF spores. The statistical test (inferential analysis) started with conducting normality and homogeneity tests (Utami & Putra, 2020) as a condition for subsequent parametric and non-parametric tests with a confidence level of 95%. The significance test results > 0.05 on the normality and homogeneity test showed that data were normally distributed and homogeneous so that a parametric test had to be done. The significance test < 0.05 on both parametric and non-parametric tests showed that the two pioneer plants comparison was significantly different, both in the percentage of spore infection and the number of spores.

Results and Discussion

AMF infection in root samples The AMF structure was found to have infected all the root samples of *C. mucunoides* and *V. unguiculata*. The AMF structures found were internal spores, internal hyphae, vesicles, and external hyphae. The highest percentage of AMF infection in the roots of *C. mucunoides* was found in areas of moderate damage (76%), while the highest percentage of AMF infection in *V. unguiculata* was found in areas with minor damage (74%) (Figure 2). AMF was identified to have infected both plants' roots in areas with heavy damage with a 66% infection rate. Based on Anova test results, the levels of damage by Mount Merapi's eruption did not significantly affect the percentage of AMF infection. This result is indicated by a significance of 0.803 for *C. mucunoides* and a significance of 0.805 for *V unguiculata*.

The number and ability of AMF to infect host plant roots are supported by several environmental parameters. Abiotic in three research locations have a value range between 22–30 °C for soil temperature, 5.2–6.9 for soil pH, 0–58% for soil moisture, 0–30° for soil moisture, 0.94–5.33% for organic carbon soil, 531–2,445 lux for light intensity, and 1–55 mm for daily rainfall. According to BMKG (2021), daily rainfall with a range of 0.5–20 mm day^{-1} includes low intensity, 20–50 mm day^{-1} includes moderate intensity, while 50–100 mm day^{-1} includes heavy intensity. The correlation test results carried out on the percentage of AMF infection on the abiotic factor showed that in the area with moderate damage, AMF infection in the roots of C. *mucunoides* was correlated with a slope with a significance of 0.025. In contrast, a minor damage area is correlated with light intensity with a significance of 0.025. On the other hand, there was no significant correlation between AMF infection in *V. unguiculata* roots and the measured abiotic factors. AMF is an obligate parasite, so the surrounding environment directly affects the host plant (Munarti et al., 2018; Rochdjatun & Sastrahidayat, 2011). The average slope of the soil at the minor and moderate damage areas was relatively high, namely 20.3° to 21.3°, in contrast to the heavy damage area with an average slope of 2°. Soil slope can affect light intensity in a forest ecosystem (Molles, 2008). Even though the density and carbon stock of trees at the area of minor and

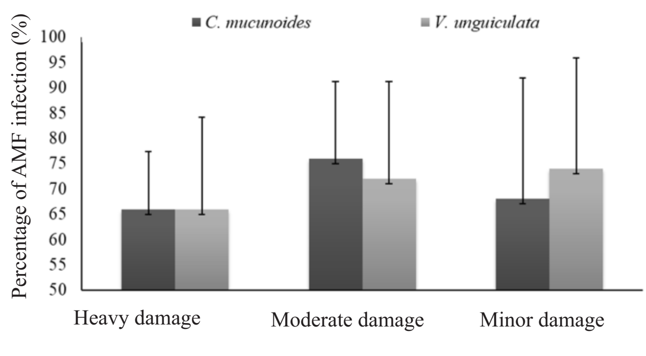

Figure 2 The percentage of AMF infection in the root samples of *C. mucunoides* and *V. unguiculata.*

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moderate damage is in the high category based on Reducing Emission from Deforestation and Forest Degradation or REDD+ (Yusuf, 2020), the average light intensity in two locations is relatively high, 1,295 lux and 1,276 lux. The average soil temperature at the minor and moderate damage areas ranges from 23°C and 26°C, with soil moisture ranging from 53% and 30%. The heavy damage area, which was still dominated by understorey plants (Husna, 2020), was recorded as having the highest light intensity, 1,873 lux, and recorded as having the highest soil temperature and the lowest soil moisture 30°C and 3%. Light affects the photosynthetic process of host plants, especially *C. mucunoides*, which live in open areas with a high to low tolerance light intensity (500–10,000 lux), can live up to 2,000 m asl, and can live at low pH (4.5–5) (Syarif et al., 2007). The altitude and slope of an area will affect the host plant's growth and influence other abiotic factors (Suharno et al., 2014; Casazza et al., 2017). Abiotic factors such as soil temperature and humidity can directly influence the abundance and species of AMF in an ecosystem (Klironomos et al., 2001; Kivlin et al., 2011). AMF can be associated with 90–95% of plant roots scattered from the Arctic to the tropics and from deserts to forests (Kandari et al., 2016). The factors differentiating between regions are AMF and their host plants (De-Souza et al., 2012). The images of internal spores, internal hyphae, vesicles, and external hyphae that infect plant root samples can be seen in Figure 3.

Number of AMF spores in the rhizosphere The presence of spores in the rhizosphere of *C. mucunoides* and *V. unguiculata* will support the abundance of AMF in areas affected by Mount Merapi's eruption. The highest number of AMF spores was found in the area with minor damage where on average, the rhizosphere of *C. mucunoides* had 10.4 spores, and the rhizosphere of *V. unguiculata* had nine spores (Figure 4). Different levels of damage by the 2010 Merapi eruption did not significantly affect the average number of spores in the rhizosphere of *C. mucunoides* and *V. unguiculata*. This result is indicated by a significance of 0.801 for *C. mucunoides* and 0.083 for *V. unguiculata*. The presence of AMF in the areas affected by the 2010 Merapi eruption shows the role of AMF in the succession process. The spores in the rhizosphere of *C. mucunoides* and *V. unguiculata* can be seen in Figure 5.

The presence of AMF spores was validated with the INVAM website data. In *C. mucunoides* and *V. unguiculata*'s rhizosphere, it can be concluded that there are five genera identified, namely *Scutellospora, Sclerocystis, Glomus, Gigaspora*, and *Acaulospora.* The spores in the genus *Scutellospora* and *Gigaspores* form a bulbous suspensor holder of hyphae, but the spores on *Scutellopspora* have an ornament germination shield (Nusantara et al., 2012). The spores in the genus *Scleriocyctis* are brown, forming a mark (sporocarp), and the outside is slippery (Tuheteru et al., 2019). Spores in the genus *Acaulospora* are light brown to dark brown and round (INVAM, 2017; Lee et al., 2018). The spores in the genus *Glomus* are oval, yellow with a slippery surface (INVAM, 2017). Genus *Glomus* dominates all study sites in both plants (Figure 6 and Figure 7), where the difference between the two plants lies in the genus that dominates the next. The rhizosphere *C. mucunoides* is further dominated by *Acaulospora* and *Scutellospora,* while in the rhizosphere, *V. unguiculata* is further dominated by *Scutellospora* and *Gigaspora*. According to Ansiga et al. (2017), the genus *Glomus, Ascaulospora*, and *Sclerocystis* were identified in other Fabaceae plants' rhizosphere

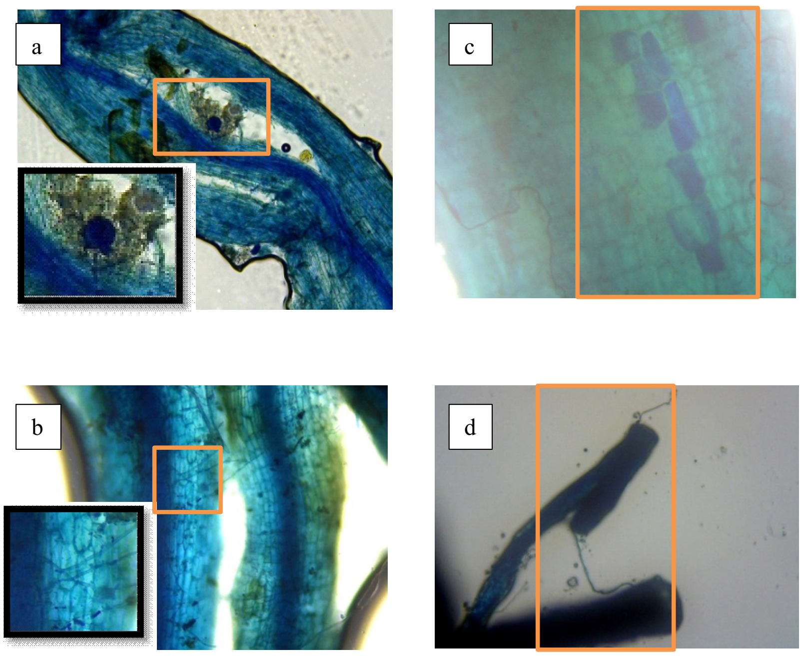

Figure 3 AMF structure in *C. mucunoides* and *V. unguiculata* from Fabaceae family with a magnification of 10: internal spores (a), internal hyphae (b), vesicles (c), external hyphae (d).

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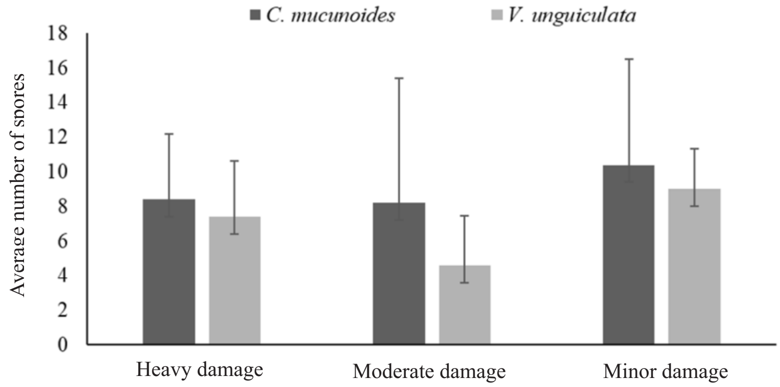

Figure 4 The average number of spores in the rhizosphere of *C. mucunoides* and *V. unguiculata.*

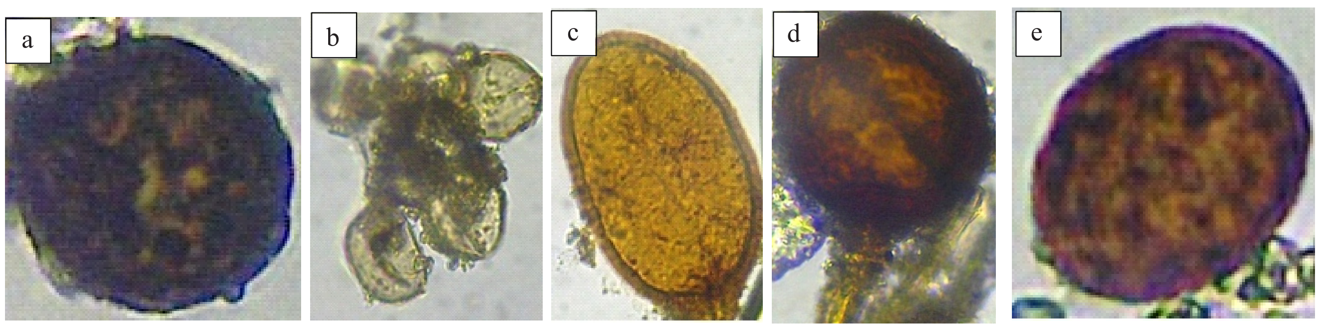

Figure 5 Spores in the rhizosphere of *C. mucunoides* and *V. unguiculata* (a–d) (magnification 100): *Scutellospora* sp. (a), *Sclerocystis* sp. (b), *Glomus* sp. (c), *Gigaspora* sp. (d), and *Acaulospora* sp. (e).

Leucaena leucocepala, *Calliandra calothyrsus,* and *Gliricidia sepium*. The number of spores in the plant rhizosphere, especially the Fabaceae family, can indicate a land's fertility (Syib'ili et al., 2013).

The results of the statistical tests showed that, in general, the average number of spores in the rhizosphere of *C. mucunoides* roots did not have any correlation with any abiotic factors, whereas *V. unguiculata* correlated with soil organic carbon with a significance of 0.016 and a correlation coefficient of 0.765*. On the other hand, the results of the correlation Pearson test at each damage level showed a correlation between the average number of spores in the root rhizosphere of *V. unguiculata* with soil pH with a significance of 0.033 and a correlation coefficient of 0.999* and a correlation with rainfall with a significance of 0.033 and a correlation coefficient of 0.999*. Environmental factors, such as soil fertility and soil moisture, also influence the spore formation process (Rainiyati, 2007). Soil fertility can be described from the carbon organic soil (COS), where the average COS at minor damage area was 4.6%, moderate damage area was 2.5%, and heavy damage area was 2.0%. AMF contributes to increasing soil organic carbon (Rillig et al., 2001; Ihsan et al., 2015). AMF not only actively participates in the carbon cycle by increasing the flow of carbon to the soil (Cardoso & Kuyper, 2006) but also has a function as a bio activator in storing carbon in the rhizosphere through the glomalin structure in its body (Driver et al., 2005; Nusantara et al., 2012). Sampling was carried out at the end of the long dry season in December 2019, with monthly rainfall starting to enter the medium intensity of 100–150 mm (Prasetyaningtyas, 2020). During data collection, the average daily rainfall at minor damage area was 5.8 mm with a soil pH of 5.4, while at moderate damage area, the average daily rainfall reached 34 mm with soil pH was 6.2. According to Odum (1993), rainfall and vegetation density above the soil surface affect soil moisture. The number of AMF spores varies according to the season, and the highest AMF colonization is during high rainfall conditions, while the highest number of spores is at the end of the dry season (Guadarrama et al., 2014). High rainfall can decrease the number of spores (Suharno et al., 2014), while fluctuations in soil moisture can affect spore formation or sporulation (Muhibuddin et al., 2014). Most AMF is acidophilic or optimally live in an environment with an

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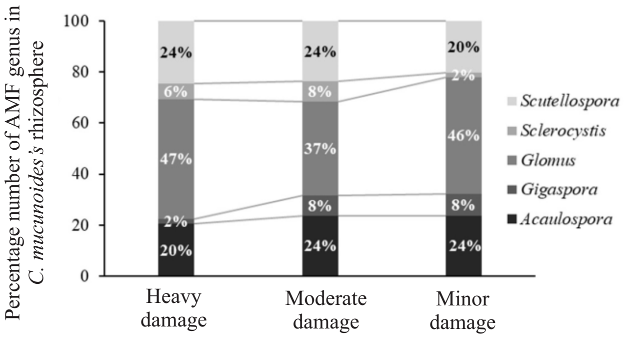

Figure 6 Percentage number of AMF genus in *C. mucunoides*'s rhizosphere.

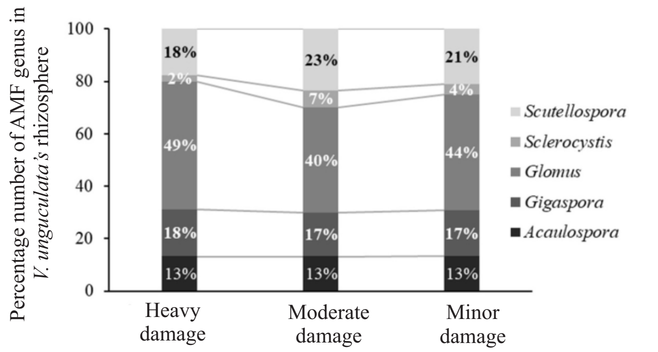

Figure 7 Percentage number of AMF genus in *V. unguiculata*'s rhizosphere.

acidic pH (Saputra et al., 2015). The genus *Glomus* develop well on soils with pH 5.6–7, the genus *Gigaspora* with 4-6, and the genus *Acaulospora* with 4–5 (Tuheteru, 2003; Margarettha, 2011). The abundance of mycorrhizal spores is influenced by climatic factors, which will affect soil characteristics, physiology of the host plant, and the mycorrhizal symbiotic relationship (Octavianti & Ermavitalini, 2014).

Conclusion

The highest mean percentage of AMF infection in *C. mucunoides* was found in the areas with moderate damage, with a percentage of 76%. In comparison, *V. unguiculata* was found in minor damage areas with a percentage of 74%. The highest number of AMF spores was found in the areas with minor damage, i.e., the rhizosphere of *C. mucunoides* had 10.4 spores, and the rhizosphere *V. unguiculata* had nine spores. Different damage levels did not significantly influence the percentage of AMF infection and the number of AMF spores. In *C. mucunoides* and *V. unguiculata*'s rhizosphere, it can be concluded that there are five genera identified, namely *Scutellospora, Sclerocystis, Glomus, Gigaspora*, and *Acaulospora,* where the genus *Glomus* dominates all study sites in both plants.

Recommendation

Knowledge of the symbiotic relationship between pioneer plants and AMF in MMNP is essential to be studied further. The types of indigenous AMF in MMNP need to be further identified, where the forest ecosystem in MMNP is an ecosystem with high eruption disturbances. The rehabilitation process at MMNP can collaborate with local pioneer plants that have been inoculated by AMF so that survival opportunities can be increase.s Future research is expected to reach a wider area of MMNP to record more types of indigenous AMF symbiosis with more various local pioneer plants in MMNP.

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